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WOOLWINE, SAMUEL C	
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Please find below and/or attached an Office communication concerning this application or proceeding.

The time period for reply, if any, is set in the attached communication.

Office Action Summary	Application No.	Applicant(s)	
	10/562,840	DRESSMAN ET AL.	
	Examiner	Art Unit	
	SAMUEL WOOLWINE	1637	

-- The MAILING DATE of this communication appears on the cover sheet with the correspondence address --

Period for Reply

A SHORTENED STATUTORY PERIOD FOR REPLY IS SET TO EXPIRE *3* MONTH(S) OR THIRTY (30) DAYS, WHICHEVER IS LONGER, FROM THE MAILING DATE OF THIS COMMUNICATION.

- Extensions of time may be available under the provisions of 37 CFR 1.136(a). In no event, however, may a reply be timely filed after SIX (6) MONTHS from the mailing date of this communication.
- If NO period for reply is specified above, the maximum statutory period will apply and will expire SIX (6) MONTHS from the mailing date of this communication.
- Failure to reply within the set or extended period for reply will, by statute, cause the application to become ABANDONED (35 U.S.C. § 133). Any reply received by the Office later than three months after the mailing date of this communication, even if timely filed, may reduce any earned patent term adjustment. See 37 CFR 1.704(b).

Status

1)☒ Responsive to communication(s) filed on *23 March 2009*.

2a)☐ This action is **FINAL**. 2b)☒ This action is non-final.

3)☐ Since this application is in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11, 453 O.G. 213.

Disposition of Claims

4)☒ Claim(s) *35-54 and 59-63* is/are pending in the application.

4a) Of the above claim(s) _____ is/are withdrawn from consideration.

5)☐ Claim(s) _____ is/are allowed.

6)☒ Claim(s) *35,36,38,40-42,46-54,59,61 and 63* is/are rejected.

7)☒ Claim(s) *37,39,43-45,60 and 62* is/are objected to.

8)☐ Claim(s) _____ are subject to restriction and/or election requirement.

Application Papers

9)☐ The specification is objected to by the Examiner.

10)☐ The drawing(s) filed on _____ is/are: a)☐ accepted or b)☐ objected to by the Examiner.

Applicant may not request that any objection to the drawing(s) be held in abeyance. See 37 CFR 1.85(a).

Replacement drawing sheet(s) including the correction is required if the drawing(s) is objected to. See 37 CFR 1.121(d).

11)☐ The oath or declaration is objected to by the Examiner. Note the attached Office Action or form PTO-152.

Priority under 35 U.S.C. § 119

12)☐ Acknowledgment is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d) or (f).

a)☐ All b)☐ Some * c)☐ None of:

1.☐ Certified copies of the priority documents have been received.

2.☐ Certified copies of the priority documents have been received in Application No. _____.

3.☐ Copies of the certified copies of the priority documents have been received in this National Stage application from the International Bureau (PCT Rule 17.2(a)).

* See the attached detailed Office action for a list of the certified copies not received.

Attachment(s)

1) ☒ Notice of References Cited (PTO-892)
2) ☐ Notice of Draftsperson's Patent Drawing Review (PTO-948)
3) ☒ Information Disclosure Statement(s) (PTO/SB/08) Paper No(s)/Mail Date *01/13/2009;04/22/2009*.
4) ☐ Interview Summary (PTO-413) Paper No(s)/Mail Date. _____ .
5) ☐ Notice of Informal Patent Application
6) ☐ Other: _____.

DETAILED ACTION

Status

Applicant's response filed 03/23/2009 is acknowledged. Presently, claims 35-54 and 59-63 are pending. Any previous rejection of a claim that has been cancelled is withdrawn as moot.

Response to Arguments

Applicant's remarks and declaration in response to the rejections based on Leamon et al (US 7,323,305) have been considered. Applicant has filed a declaration under 37 CFR 1.131 to antedate the Leamon patent. The Leamon patent claims priority to seven provisional applications. The date prior to which Applicant's declaration purports to show invention is June 6, 2003. This would therefore antedate all but two of the provisional applications to which Leamon claims priority, and would necessarily antedate the filing date of the patent itself (whether January 8, 2004 which filing date has been accorded, or September 22, 2004 as shown on the face of the patent as originally issued). Therefore, the relevant issues to decide are:

1. Does the subject matter of either of the two earlier provisional applications of Leamon anticipate (or render obvious) any of Applicant's claims?

2. Are any of Applicant's claims drawn to the same invention claimed in any claim of the Leamon patent?

3. Has Applicant's declaration demonstrated reduction to practice for each of Applicant's claims prior to June 6, 2003?

With regard to question 1, provisional application 60/443,471 does not teach or suggest emulsions or microemulsions. Therefore this provisional application is irrelevant. Provisional application 60/465,071 has been reviewed. While the material in this provisional application does not anticipate Applicant's remaining claims, some claims are obvious over the material presented therein. Accordingly, new grounds of rejection are set forth below, and this action is NON-FINAL.

With regard to question 2, none of Applicant's claims are drawn to the same invention as claimed in the Leamon patent.

With regard to question 3, Applicant's declaration does not evidence reduction to practice of claims 38, 40, 54 or 61.

Claims 38 and 61 recite "recovering the first species of analyte DNA molecule from the product beads." Applicant's declaration cites to the paragraph spanning pages 8821-2 of the Dressman publication (PNAS 100(15):8817-22, July 22, 2003) to provide basis for claims 38 and 61 (declaration items 8 and 27). This passage indicates that beads with immobilized DNA molecules were used as *templates* in a PCR reaction, such that *amplified copies* of the "first species of analyte DNA molecule" were produced. However, the claims literally recite recovering the DNA molecule *from* the bead, which was not done in the Dressman publication. While the language of claims 38 and 61 could be creatively construed such that amplifying the bead bound DNA is "recovering the first species of analyte DNA molecule from the product beads", the claim language clearly encompasses the more literal meaning, i.e. removing the DNA molecules from the beads. Applicant has not shown reduction to practice of this

element. Because claim 40 depends from claim 38, this claim has also not been shown to have been reduced to practice prior to June 6, 2003.

Claim 54 recites "wherein the step of determining a sequence feature is performed by extension of a primer with one or more labeled deoxyribonucleotides". Claim 35, from which claim 54 depends, recites "determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads" (emphasis provided). Applicant cites to page 8818, column 2, second paragraph of the Dressman publication to provide basis for claim 54 (declaration item 24). The cited passage describes that "PCR products to be used as templates for BEAMing or sequencing...". This does not indicate the primer extension sequencing of a DNA molecule which is bound to the product beads. In addition, the paragraph spanning pages 8821-2 makes clear that it was PCR products generated from the bead-bound DNA molecules, not the bound molecules themselves, which were sequenced.

Because the declaration does not evidence reduction to practice of the method of claims 38, 40, 54 or 61, the rejection of these claims may rely on the support of Leamon's provisional application 60/476,504 (filed June 6, 2003). The rejection of the remaining claims may rely only on teachings supported by Leamon's provisional application 60/465,071 (filed April 23, 2003).

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

A person shall be entitled to a patent unless –

> (e) the invention was described in (1) an application for patent, published under section 122(b), by another filed in the United States before the invention by the applicant for patent or (2) a patent granted on an application for patent by another filed in the United States before the invention by the applicant for patent, except that an international application filed under the treaty defined in section 351(a) shall have the effects for purposes of this subsection of an application filed in the United States only if the international application designated the United States and was published under Article 21(2) of such treaty in the English language.

Claims 38, 40 and 61 are rejected under 35 U.S.C. 102(e) as being anticipated by Leamon et al (US 7,323,305, priority date for this rejection: June 6, 2003 based on provisional application 60/476,504). The rejection has not been applied to independent claims 35 and 59 based on Applicant's declaration showing prior invention.

With regard to claims 38, 40 and 61, Leamon teaches a method comprising:

forming microemulsions comprising one or more species of analyte DNA molecules;

See claim 1: "delivering the fragmented nucleic acids into aqueous microreactors in a water-in-oil emulsion such that a plurality of aqueous microreactors comprise a single copy of a fragmented nucleic acid".

amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule;

See claim 1: "amplifying the fragmented nucleic acids in the microreactors to form amplified copies of said nucleic acids and binding the amplified copies to beads in the microreactors". See figure 6, and column 20, line 28 through column 21, line 20.

separating the product beads from analyte DNA molecules which are not bound to product beads;

See figure 1G, at "Sequencing" step, "Second Strand Removal".

determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads.

See claim 1: "performing a sequencing reaction simultaneously on a plurality of the reaction chambers".

See also columns 20-21 and figure 6, and provisional application 60/476,504 pages 4-5 and figure 2.

With regard to claims 38, 40 and 61, see column 24, lines 46-54: "Alternatively, the beads may be isolated and the DNA may be removed from each bead and sequenced." See also page 14, line 10 of Leamon provisional application 60/476,504.

Claim Rejections - 35 USC § 103

The following is a quotation of 35 U.S.C. 103(a) which forms the basis for all obviousness rejections set forth in this Office action:

> (a) A patent may not be obtained though the invention is not identically disclosed or described as set forth in section 102 of this title, if the differences between the subject matter sought to be patented and the prior art are such that the subject matter as a whole would have been obvious at the time the invention was made to a person having ordinary skill in the art to which said subject matter pertains. Patentability shall not be negatived by the manner in which the invention was made.

This application currently names joint inventors. In considering patentability of the claims under 35 U.S.C. 103(a), the examiner presumes that the subject matter of the various claims was commonly owned at the time any inventions covered therein were made absent any evidence to the contrary. Applicant is advised of the obligation under 37 CFR 1.56 to point out the inventor and invention dates of each claim that was not commonly owned at the time a later invention was made in order for the examiner to

consider the applicability of 35 U.S.C. 103(c) and potential 35 U.S.C. 102(e), (f) or (g) prior art under 35 U.S.C. 103(a).

Claim 54 is rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: June 6, 2003 based on provisional application 60/476,504) in view of Taing et al (US 2002/0102590).

With regard to claim 54, Leamon teaches a method comprising:

forming microemulsions comprising one or more species of analyte DNA molecules;

See claim 1: "delivering the fragmented nucleic acids into aqueous microreactors in a water-in-oil emulsion such that a plurality of aqueous microreactors comprise a single copy of a fragmented nucleic acid".

amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads, wherein the reagent beads are bound to a plurality of molecules of a primer for amplifying the analyte DNA molecules, whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule;

See claim 1: "amplifying the fragmented nucleic acids in the microreactors to form amplified copies of said nucleic acids and binding the amplified copies to beads in the microreactors". See figure 6, and column 20, line 28 through column 21, line 20.

separating the product beads from analyte DNA molecules which are not bound to product beads;

See figure 1G, at "Sequencing" step, "Second Strand Removal".

determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads.

See claim 1: "performing a sequencing reaction simultaneously on a plurality of the reaction chambers".

See also columns 20-21 and figure 6, and provisional application 60/476,504 pages 4-5 and figure 2.

With regard to the limitations specifically recited in claim 54, see column 24, lines 46-62 where Leamon teaches that the amplified DNA on the bead may be sequenced directly on the bead by transferring the bead to a reaction vessel and subjecting to pyrophosphate or Sanger sequencing. See also provisional application 60/476,504 page 14, lines 5-15.

Provisional application 60/476,504 does not teach "extension of a primer with one or more labeled nucleotides".

Taing teaches (paragraph [0006]):

"In DNA sequencing, it is now conventional to use two or more (usually four) different fluorescent labels to distinguish sequencing fragments that terminate with one of the four standard nucleotide bases (A, C, G and T, or analogs thereof). Such labels are usually introduced into the sequencing fragments using suitably labeled extension primers (dye -primer method) or by performing primer extension in the presence of nonextendable nucleotides that contain unique labels (Sanger dideoxy terminator method)."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention of claim 54 was made to carry out extension of a primer with one or more labeled deoxyribonucleotides when practicing Leamon's method, because Leamon teaches Sanger sequencing and Taing teaches that Sanger sequencing involves extension of a primer with one or more labeled deoxyribonucleotides.

Claims 35, 36, 46, 47, 49, 59 and 63 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000).

As the rejection may only rely on the subject matter disclosed in the '071 provisional application, the relevant passages from this provisional application are reproduced below:

Pages 46-47:

A second approach to amplifying and capturing both strands will be to amplify the fragment library offline in a single tube using oil and surfactant-based emulsions to encapsulate the capture beads, template and PCR reaction mix. This approach will maintain the clonality of the amplification, provide

a single-tube format for second strand removal, sequencing primer annealing and the addition of signal-producing enzymes. The average size of the emulsion capsules must be optimized to maximize the number of single beads containing single strands of DNA, that can be incorporated within a single emulsion volume. An adequate volume-to-bead ratio must be maintained in order to insure a maximum number single bead capsules. Consequently we anticipate that the emulsion volumes must continue to increase to allow for capture of increasingly larger numbers of fragments from larger genomes. The single, large volume of the emulsion PCR mix can be aliquoted into standard PCR tubes and all aliquots run simultaneously in a standard thermalcycler. Bead-to-emulsion volume ratios must be adequate to accommodate a balance between total volume and absolute bead numbers. Smaller genomes can be amplified in single PCR tubes allowing for many whole genome libraries to be amplified at the same time. We will attempt to limit the total volume of emulsion without expanding the amplification effort beyond a full 96-well thermalcycler.

Sequencing.

Each bead is covalently loaded with large numbers of two oligonucleotides complementary to the 3' ends of our two universal linker sequences found on each strand of the amplified product. We will specifically capture the single stranded forms of the complementary strands of an amplified DNA fragment by using these two oligonucleotides. These oligonucleotides are each targeting the 3' end of their respective stands. Both capture oligonucleotides are conjugated to the solid phase capture bead resulting in the simultaneous capture of the many copies of each strand on the same single bead in a single well. In turn, we must then anneal sequencing primers to these fragments and perform our initial sequencing reaction to obtain 50 to 100 bases of sequence from one strand.

Page 40:

Genomic DNA is fragmented and delivered to the wells via dilution. The wells contain separate primer beads (blue beads) on which double stranded fragments are captured after PCR with one strand of the template covalently attached to the bead. The second strand (non-covalently attached) is physically removed and the DNA sequencing primer is hybridized to the5' end of the remaining strand.

Page 38:

The process used on the 454 Corporation instrument is:

- Isolate the DNA,
- Enzymatic fragmentation of the DNA, and adaptor ligation,
- Delivery of the adapted DNA fragments via fluidics to reaction wells,
- Solid-phase amplification of the isolated DNA fragments within each well,
- Solid phase DNA sequencing on the resulting amplicons within each well,
- Massively parallel sequencing data collection for each fragment,
- Novel assembly of the short read length fragments into a whole genome.

With regard to claim 35, the text from pages 46-47 teaches:

forming microemulsions comprising one or more species of analyte DNA molecules and *amplifying analyte DNA molecules in the microemulsions in the presence of reagent beads* ("amplify the fragment library offline in a single tube using oil and surfactant-based emulsions to encapsulate the capture beads, template and PCR reaction mix");

wherein the reagent beads are bound to a plurality of molecules of a primer ("Each bead is covalently loaded with large numbers of two oligonucleotides complementary to the 3' ends of our two universal linker sequences found on each strand of the amplified product.");

whereby product beads are formed which are bound to a plurality of copies of one species of analyte DNA molecule ("We will specifically capture the single stranded forms of the complementary strands of an amplified DNA using these two

oligonucleotides. These oligonucleotides are each targeting the 3' end of their respective strands.");

separating the product beads from analyte DNA molecules which are not bound to product beads ("This approach will maintain the clonality of amplification, provide a single-tube format for second strand removal, sequencing primer annealing and the addition of signal-producing enzymes." From the context it is clear that "second strand removal" would remove any DNA not bound to the beads.);

determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads ("we must then anneal sequencing primers to these fragments and perform our initial sequencing reaction to obtain 50 to 100 bases of sequence from one strand.").

With regard to claim 47, Leamon teaches genomic DNA (see cited text from page 40 of the '071 provisional application above).

With regard to claim 49, the emulsion amplification/capture technique discussed on pages 46-47 of the '071 provisional would necessarily produce PCR products from genomic fragments.

With regard to claim 36 it would have been obvious to separate the beads into different wells of the sequencing apparatus disclosed in 60/465,071 after the amplification and capture steps (see page 47: "Both capture oligonucleotides are conjugated to the solid phase capture bead resulting in the simultaneous capture of the many copies of each strand on the same single bead in a single well."). It would have been realized by one of ordinary skill that, after the emulsion amplification/capture

(described in the passage spanning pages 46-47) the resulting heterogeneous population of beads would have to be separated into individual beads in discrete wells in order for the subsequent sequencing to occur. Performing the sequencing reaction in a well with multiple beads, each with a different template attached, would not have been feasible, as would have been understood by one of ordinary skill in the area of DNA sequencing technology.

With regard to claim 59, the only difference over claim 35 is the lack of a step of *determining a sequence feature of the one species of analyte DNA molecule which is bound to the product beads* and the addition of the limitations recited in claim 36. Therefore, what has been discussed for claims 35 and 36 applies to claim 59 as well.

Similarly, claim 63 merely adds the limitation of *determining the sequence of the first species of analyte DNA molecule*, which limitation was discussed for claim 35.

The only limitation of claim 35 not provided for is the limitation *a primer for amplifying the DNA molecules*. Page 44 of the provisional application (Figure 7 and caption, discussing an embodiment where amplification and capture take place in a well, not in an emulsion) teaches denaturing double stranded fragments and hybridizing to oligonucleotides on the capture beads:

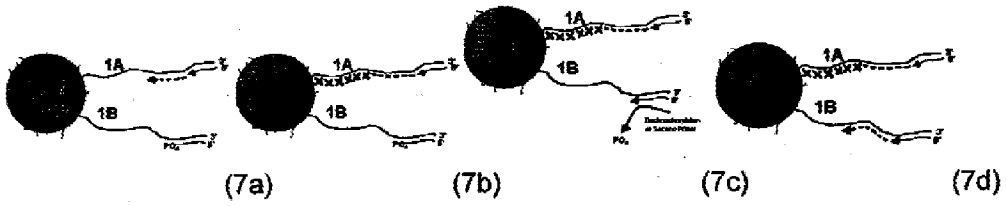

Figure 7. Double-Ended Sequencing process:

In a single well, millions of copies of single amplified fragment are produced. (7a) Denaturation of the double stranded fragments allows for simultaneous hybridization of both strands (labeled 1A and 1B) to occur to their respective capture oligonucleotides on the bead. Sequencing primers specific to the 5' end of their respective strands are annealed simultaneously. The second strand (1B) sequencing primer containing a 3' PO4 to prevent extension by the DNA polymerase. Sequencing by synthesis is performed as described. (7b) First strand (1A) sequencing is terminated and the template rendered inactive. (7c) The 3' phosphate or other blocking moiety is removed from the second strand sequencing primer to activate it as a substrate for the DNA polymerase. (7d) Sequencing by synthesis on the second strand (1B) is initiated and completed as described.

The discussion on pages 46-47 does not elaborate on whether this is how the amplified DNA is captured onto the beads in the emulsion format. It is noted that, unless the oligonucleotides immobilized on the beads were somehow blocked (a point on which the discussion is silent), extension of the immobilized oligonucleotides would inherently have occurred during the emulsion amplification, since "These oligonucleotides are each targeting the 3' end of their respective strands." That is, the oligonucleotides were of the proper orientation such that they would have been extended unless they were specifically blocked (e.g. synthesized to comprise a 3' terminal phosphate, amino group or other moiety to prevent extension). Again, the discussion in the '071 provisional makes no statements to this effect.

Elsewhere (pages 38 and 40), the '071 provisional makes reference to "solid-phase amplification" and "primer beads", but this is not in the context of the emulsion amplification/capture technique discussed on pages 46-47.

Leamon also does not teach the combination of bead-bound primers and other primers not bound to the beads, as recited in claim 46.

With regard to claims 35 and 46, Andreadnis teaches a solid-phase PCR method for simultaneously amplifying and immobilizing nucleic acid templates to beads, wherein a combination of bead-immobilized and solution phase primers (which meets the limitations of claim 46) were used to generate covalently attached amplicons by use of the immobilized primers in the amplification process (see entire article, for example Abstract). In discussing previous applications of immobilized PCR products in the prior art, Andreadnis teaches (page i, beginning of last paragraph): "Although the utility of these techniques is clear, they still involve initial PCR amplification and subsequent immobilization of the PCR products. To improve the efficiency of this technology, PCR amplification and immobilization can be combined into a single step...Using our methodology, bead-bound primers are used to simultaneously amplify and covalently immobilize one or more DNA amplicons...".

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to modify the methods of Leamon (supported by provisional application 60/465,071) by utilizing the oligonucleotides attached to the beads as primers (as taught by Andreadnis) in the emulsion amplification. As the oligonucleotides on Leamon's beads were already of the proper sequence and orientation, all that would have been needed was to not *prevent* the extension of these primers during the amplification (which prevention Leamon never discussed doing in any case). One would have been motivated to make this modification since Andreadnis taught this would improve efficiency over performing the amplification and *then* immobilizing the PCR product. In addition, it would have been understood by one of

skill in the art that this modification would have resulted in covalent attachment, rather than attachment based on denaturing the double-stranded amplification product and then trying to hybridize the denatured strands to the oligonucleotides on the beads. One of skill in the art would have realized that such covalent attachment would have resulted in a stronger association of the captured strand to the bead, which would in turn have facilitated the "second strand removal" discussed on pages 46-47 of Leamon's provisional disclosure.

Claim 42 is rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000) as applied to claims 35, 36, 46, 47, 49, 59 and 63 above and further in view of Becker (U.S. Patent 5,546,792).

The teachings of Leamon and Andreadnis have been discussed.

With regard to claim 42, Leamon does not teach breaking emulsions by adding a detergent.

Becker teaches (Example II, columns 6-7) an example of using detergent to break down an oil and water emulsion.

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use detergents to break the emulsion when practicing Leamon's methods as it was already known in the art that detergents could be used to

break emulsions. One would have been motivated to break the emulsion down in order to recover the beads following the emulsion amplification/capture.

Claims 48 and 50 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000) as applied to claims 35, 36, 46, 47, 49, 59 and 63 above and further in view of Bamber et al (US 2003/0065144).

The teachings of Leamon and Andreadnis have been discussed.

With regard to claims 48 and 50, Leamon did not discuss sequencing cDNA or PCR products thereof.

Bamber teaches (paragraph [0182]): "PCR reactions using reverse-transcribed polyA-selected RNA (see `Preparation of first-strand cDNA` section) or purified genomic DNA for sequencing (see ` Genomic Sequencing`) were performed as follows..."

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the sequencing method suggested by the combined teachings of Leamon and Andreadnis to the sequencing of cDNA or PCR products thereof, as Bamber shows that it was known in the art, and therefore of interest to one of skill in the art, to sequence these types of nucleic acids.

Claim 53 is rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional

application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000) as applied to claims 35, 36, 46, 47, 49, 59 and 63 above and further in view of Macevicz (U.S. Patent 6,306,597).

The teachings of Leamon and Andreadnis have been discussed.

With regard to claim 53, Leamon does not teach that the beads used in the emulsion amplification are magnetic beads.

Macevicz teaches attaching amplified target polynucleotides to magnetic beads "for ease of separating the target polynucleotide from other reagents used in the method" (column 8, lines 30-35).

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to use magnetic beads in the method suggested by the combined teachings of Leamon and Andreadnis, since it was known in the prior art to use magnetic beads as solid supports for polynucleotides to facilitate their separation from other reagents used in nucleic acid methods as taught by Macevicz.

Claims 51 and 52 are rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000) as applied to claims 35, 36, 46, 47, 49, 59 and 63 above and further in view of Zarling et al (US 2003/0105039).

The teachings of Leamon and Andreadnis have been discussed.

With regard to claims 51 and 52, Leamon did not elaborate on whether the sequencing method was to be applied to a "single individual" or a "population of individuals".

Zarling teaches (paragraph [0049]; see also figure 21): "Sequence analysis of exon4 of the mouse OTC gene in founder mice. PCR amplification of genomic DNA from tail biopsies of a pool of all of the homozygous (spf-ash/spf-ash) females used as egg donors and each indicated individual founder mice were sequenced using cycle sequencing…".

It would have been *prima facie* obvious to one of ordinary skill in the art at the time the invention was made to apply the sequencing method suggested by the combined teachings of Leamon and Andreadnis to either or both of "individuals" or "populations" (i.e. pools) of individuals, since Zarling demonstrated that there were clearly situations where sequencing of individuals and populations would be desired.

Claim 41 is rejected under 35 U.S.C. 103(a) as being unpatentable over Leamon et al (US 7,323,305, priority date for this rejection: April 23, 2003 based on provisional application 60/465,071) in view of Andreadnis et al (Nucleic Acids Research 28(2):e5(i-viii), 2000) as applied to claims 35, 36, 46, 47, 49, 59 and 63 above and further in view of Williams (US 2003/0064400).

The teachings of Leamon and Andreadnis have been discussed. These references did not teach or suggest that less than 10% of the reagent beads are converted to product beads.

It is clear, however, that Leamon intended to amplify a single fragment and attach the products to a single bead (see page 47 of provisional application 60/465,071: "The average size of the emulsion capsules must be optimized to maximize the number of single beads containing single strands of DNA..."). Leamon is silent with respect to a discussion of the relative concentrations of beads and templates, and the consequent percentage of beads that would undergo conversion to product beads (i.e. in Leamon's method, a reagent bead would be converted to a product bead only if that bead were contained in an emulsion droplet that also contained one or more templates).

Williams teaches a method for obtaining a single bead with a single nucleic acid attached thereto (see paragraph [0022] and figure 3). Williams teaches that: "DNA fragment types BD, BB and Bx attach to streptavidin-coated magnetic beads under conditions of concentration and time where only some of the beads conjugate to a DNA fragment while most of the beads fail to conjugate. For example, at 1% coupling efficiency, 0.99% of the beads will have one DNA fragment while only 0.005% of beads will have more than one fragment (Poisson statistics)."

It would have been *prima facie* obvious to one of ordinary skill in the art when practicing the method suggested by the combined teachings of Leamon and Andreadnis that in order to maximize the number of beads associated with a single DNA fragment (as opposed to multiple DNA fragments), the Poisson distribution dictates that an excess of beads to DNA fragments must be used. Just as was the case in Williams' example, this would have resulted in most beads not having any associated DNA fragment (i.e. most emulsion droplets containing a bead would not contain any DNA

template fragment). Hence, the relative concentration ratio of beads to DNA fragments would have been seen as a results-effective variable, subject to routine optimization in order to maximize the number of beads bearing a single species of DNA. This optimization would have been reflected in variable amounts of unconverted beads. Williams taught only a 1% "conversion", which is within the "less than 10%" recited by the claims. As set forth by the court: "[W]here the general conditions of a claim are disclosed in the prior art, it is not inventive to discover the optimum or workable ranges by routine experimentation." *In re Aller*, 220 F.2d 454, 456, 105 USPQ 233, 235 (CCPA 1955). By optimizing the ratio of beads to DNA in order to maximize the number of beads bearing a single DNA species, one would have arrived at less than 10% of reagent beads being converted to product beads, as would have been predicted according to Poisson statistics.

Conclusion

Claims 37, 39, 43-45, 60 and 62 are objected to as being dependent upon a rejected base claim, but would be allowable if rewritten in independent form including all of the limitations of the base claim and any intervening claims.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to SAMUEL WOOLWINE whose telephone number is (571)272-1144. The examiner can normally be reached on Mon-Fri 9:00am-5:00pm.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Gary Benzion can be reached on (571) 272-0782. The fax phone number for the organization where this application or proceeding is assigned is 571-273-8300.

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/Samuel Woolwine/
Examiner, Art Unit 1637